March 30, 2001

VETERINARY SERVICES MEMORANDUM NO. 800.81

Subject: Bursal Disease Vaccine of Chicken Bursa Origin

To: Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

This memorandum gives guidance to licensees, permittees, and applicants concerning the production of Bursal Disease Vaccine, Killed Virus. The Center for Veterinary Biologics (CVB) considers license applications for this vaccine produced from any variant or standard strain(s) of infectious bursal disease virus (IBDV) propagated in chicken bursas. This memorandum specifies production recommendations and test procedures to ensure the purity of products which contain IBDV propagated in chicken bursas.

II. CANCELLATION

This memorandum cancels Veterinary Biologics Memorandum No. 800.81 dated January 18, 2000.

III. BACKGROUND

APHIS has published the Standard Requirement for Bursal Disease Vaccine, Killed Virus, in 9 CFR 113.212. This regulation requires that the vaccine "shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs." APHIS has granted an exemption to this requirement under 9 CFR 113.4(a) because some firms encountered difficulties in producing vaccines with some isolates by the standard methods.

This exemption permits the preparation of bursal disease vaccines by propagating an isolate of IBDV in chickens and then harvesting the bursal tissue from such infected chickens for the vaccine. However, this production method places these products at an increased risk of contamination with extraneous agents which may infect the chicken prior to or during production. APHIS has granted this exemption for the production of either variant or standard strains of IBDV virus in chicken bursas provided firms address this concern by establishing production procedures that ensure the purity of their product. This is under 9 CFR 113.4(b).

The January 18, 2000 version of this memorandum is being replaced after it was brought to the attention of the CVB that source flocks serologically positive for Chicken Anemia

Virus (CAV) are frequently being used in the production of bursal origin IBDV due to the demand for large numbers of birds for the production of this product. The requirement that source flocks remain seronegative to antibodies to CAV for 3 weeks following the selection of the chickens for use in vaccine production has been dropped. Currently, the CVB is reviewing the purity regulations for poultry products. While this review is not complete, the CAV status of the source flock and CAV testing of final product will be addressed in a future Proposed Rule. Input from the biologics industry on the most feasible means for such testing would be welcomed.

IV. PRODUCTION RECOMMENDATIONS

Since current Standard Requirements for virus products (9 CFR 113.200 and 113.300) are insufficient to detect chicken anemia virus (CAV) contamination and the production of bursal disease vaccine in chicken bursa presents a significant risk of such contamination, firms should use the following production procedures to ensure the purity of this vaccine when produced in chickens.

A. Master Seed Virus

Until a standard assay can be established, firms should test all avian Master Seed Viruses for extraneous CAV according to one of the optional test procedures described in Veterinary Services Memorandum No. 800.89, section IV. A. Firms may obtain test methods and training on the PCR test for CAV from the Center for Veterinary Biologics-Laboratory upon request.

B. Chickens

Firms should use only infectious bursal disease susceptible chickens derived from a specific-pathogen-free (SPF) source flock (as defined in Veterinary Services Memorandum No. 800.65) for vaccine production. Prior to their use in production, firms should house and handle chickens in isolation in order to maintain their pathogen-free status. Firms should also prevent exposure to avian pathogens through feed or transport. Since all SPF flocks are not necessarily free of CAV, firms should document that their IBDV inactivation procedure will inactivate extraneous CAV, or alternately, the firm should test the bulks prior to inactivation for the presence of CAV by a test acceptable to APHIS (specifics outlined below).

C. Validation of Inactivation Procedures

Because the risk of contamination with CAV is high and the virus can replicate in bursal tissue, each firm should also provide documentation that the inactivation procedure described in their filed Outline of Production is capable of inactivating CAV as follows:

1. *Develop a Protocol* - Each firm should develop a protocol for validation testing that is appropriate for the specific inactivation procedure defined in its Outline of Production according to the following guidelines:

a. Inoculate SPF chicks from CAV-seronegative flocks of the age specified in the Outline of Production for virus propagation with a dose of CAV sufficient to meet the validity requirements in section e below.

(1) Collect and pool bursa from these birds at 7 to 9 days post inoculation (CAV-infected bursal pool).

(2) Collect and pool bursa from uninoculated control birds of the same age and hatch (uninfected bursal pool).

(3) Use a sufficient number of birds in each group to be representative of small scale production.

b. Subject the infected and uninfected bursa pools to the physical processing procedures (homogenization, sonication, etc.) described in the Outline of Production.

c. Subject one half of the processed CAV-infected bursal pool to the inactivation procedure described in the Outline of Production (test sample). Do not inactivate the other half of the processed CAV-positive pool (positive control sample). Subject the processed uninfected bursal pool to the inactivation procedure described in the Outline of Production (negative control sample).

d. Evaluate the test sample, positive control sample, and the negative control sample for the presence of live CAV by titration in MDCC-MSB1 cells.

(1) Subculture each dilution in the titration every 48-72 hours over a 3-week period (8-12 passages each).

(2) Score each dilution as positive or negative based on the presence or absence of cytopathology.

(3) Confirm any induction of CPE using a CAV-specific antibody reagent (virus neutralization or fluorescent antibody techniques) on a representative number of cultures.

e. For a valid test, the positive control sample should have a CAV titer of at least 10^5 TCID50/ml, and the negative control sample should be negative for CAV.

f. A satisfactory test should demonstrate complete inactivation of the CAV in the test sample.

2. *Submit Protocol* - Firms should submit their protocols to the Center for Veterinary Biologics-Licensing and Policy Development for review and comment before initiating the study.

3. *Test Site* - Firms may conduct studies to validate their inactivation procedures in the firm's research facilities if they are separate and apart from production facilities or at an off-site research facility thereby preventing contamination of licensed production facilities with CAV.

4. *Changes in Inactivation Procedures* - Should the results of the firm's inactivation study indicate the need to change the method of inactivation for the product, CVB will accept changes in the method of inactivation in the Outline of Production based on data demonstrating the new procedure is adequate to inactivate CAV and successful potency assays on three consecutive production serials of the newly formulated product according to 9 CFR 113.212(d)(2).

5. *Changes to Outline* - If data to accept the inactivation procedure are accepted, the firm should document the acceptance (including the date of acceptance) in section IV. A. of each affected Outline of Production.

D. Serial Testing Option

If a firm is not able to demonstrate that its method of inactivation for the product is adequate to inactivate CAV as described in section IV. C. above or if it has not completed such testing, the firm may use the option of demonstrating the purity of its product by testing samples of each serial of vaccine prior to adding the killing agent by one of the CAV test procedures described in Veterinary Services Memorandum No. 800.89, section

IV. A. Firms using this option must designate specific test requirements in section V. A. of their Outlines of Production for such products and provide data demonstrating the validity and sensitivity of their test procedure. The acceptance of the test method studies along with the acceptance date should be documented in section V. A. along with the test.

V. ADDITIONAL TESTING RECOMMENDATIONS

A. Vaccines Produced in Facilities Producing Other Avian Vaccines

The production of Bursal Disease Vaccine in chickens in facilities that also produce other avian vaccines presents a risk of contamination of the final product due to the possible exposure of the production birds with other vaccine viruses in the production facilities. Firms should therefore test bulk or final containers of completed product produced under such conditions for purity according to the requirements found in 9 CFR 113.212 and by the following supplemental test:

1. *Inoculation of Birds* - Inoculate bulk or final container samples of completed product from each serial into 10 SPF chickens, 21 to 28 days of age, by the recommended route, using twice the recommended dose. Three to four weeks later, inoculate each bird a second time, using twice the recommended dose. (Due to the doubled volumes of these inoculations, you may divide each inoculation into more than one site.) Hold five chickens of the same source and hatch as negative controls.

2. *Test for Antibodies to Extraneous Agents* - Collect serum from each bird prior to the first inoculation and 2 to 3 weeks following the second inoculation.

a. Test individual sera for the presence of antibodies to all the infectious agents of poultry authorized for use within the licensed production facility or within any facility housing the chickens.

b. Use the serological test methods specified in the filed Outline of Production or a special outline. (Please note that ELISA assays may yield false positive results for some antigens in birds receiving oil adjuvants. Therefore, such assays may not be the test of choice for inclusion in section V of the outline.)

c. A serial which induces a positive response to any of these agents, with the exception of the bursal vaccine virus or viruses, is unsatisfactory.

B. Vaccines Produced in Isolation Facilities

If chickens used to produce Bursal Disease Vaccine are housed in isolation facilities and the production procedures used are found by the Center for Veterinary Biologics-Inspection and Compliance to be satisfactory to prevent contamination of the product with other avian viruses, CVB will not require firms to conduct supplemental testing on each serial of product as described in section V. A. above.

VI. OUTLINES OF PRODUCTION

All Outlines of Production for Bursal Disease Vaccine, Killed Virus, of chicken bursa origin should conform with the above guidelines.

/s/ Chester A. Gipson for

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